Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Abstract 2607

The Human RAP1 and GFAPε proteins increase γ-secretase activity in a yeast model system

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Objective: Alzheimer's disease (AD) is an age-related disorder that results in progressive cognitive impairment and memory loss. Deposition of amyloid β (Aβ) peptides in senile plaques is a hallmark of AD. γ-secretase produces Aβ peptides, mostly as the soluble Aβ40 with fewer insoluble Aβ42 peptides. The rare, early-onset AD (EOAD) occurs in individuals under 60 years of age. Most of the EOAD cases are due to unknown genetic causes, but a subset is known to be due to mutations in the genes encoding the amyloid precursor protein that is processed into Aβ peptides or the presenilins (PS1 and PS2) that process APP. RAP1(TERF2IP) is a telomeric protein that is responsible for maintaining genome stability. As cells replicate/age, its level decreases. It is found in cytoplasm as well as in nucleus. It's role in cytoplasm is unknown. Our study was designed to identify the interacting proteins of RAP1 and investigate its possible role in age-related diseases.

Methods: Identifying the interacting protein of RAP1 was done utilizing a yeast 2-hybrid screen. Interactions of various proteins were verified using *in vitro* co-immunoprecipitation. We modified and improved a reconstituted γ-secretase system in *Saccharomyces cerevisiae* to measure the γ-secretase activity levels. Aβ peptide production was measured using ELISA. All interactions were verified by using immunoblotting along with immunofluorescence microscopy techniques.

Results: We have identified GFAPε (glial fibrillary acidic protein-ε) as a protein that interacts with the telomere protection factor RAP1. RAP1 can also interact with PS1 alone or with both PS1 and GFAPε together *in vitro*. GFAPε co-precipitated with RAP1 from human cell extracts. RAP1, GFAPε and PS1 all co-localized in human SH-SY5Y cells. Using a γ-secretase system reconstituted in yeast, we found that RAP1 increased γ-secretase activity, and this was further increased by the co-expression of GFAPε. However, expression of GFAPε alone was not able to significantly affect γ-secretase activity. Conclusion: Our data show that the nuclear protein RAP1 has an extratelomeric role in the cytoplasm through its interactions with GFAPε and PS1. RAP1 increased γ-secretase activity, and this was potentiated by GFAPε. Our studies are the first to connect RAP1 with an age-related disorder.

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Abstract 2610

Modifications in SARS-CoV-2 N Linker Region Regulate RNA Interactions and Phase Separation

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The N protein of the SARS-CoV-2 virion is critical for viral genome packaging via RNA binding and regulation of viral transcription at the replication-transcription complex (RTC). The N protein can be divided into five main domains, and the central region is the linker, which is predicted to be primarily disordered and has not been heavily studied. The linker is Serine-Arginine Rich, which is phosphorylated at multiple sites by host kinases during infection, thereby promoting the N protein's role in viral transcription. Phosphorylation is a critical process for the regulation of many cellular processes and can provide recognition sites for binding complexes. In a study that examined the recognition of the SARS-CoV-2 N protein by the human 14-3-3 protein, the linker was found to contain critical phosphosites for 14-3-3 binding. The goals of this project are to determine the structure, dynamics, and RNA interactions of the Serine-Arginine Rich linker region. To accomplish this, we performed Nuclear Magnetic Resonance spectroscopy (NMR) experiments to analyze the structure of the linker region of the N protein and its ability to bind viral RNA. NMR confirms predictions that the linker is not entirely unstructured and it is able to bind RNA. The linker region of the N protein with phosphoserine incorporated at S188 was also examined via an NMR titration experiment with 1-1000 RNA. Compared to wild type, the incorporation of phosphorylation decreases binding. Other biophysical techniques such as Analytical Ultracentrifugation (AUC) and Multi-Angle Light Scattering (MALS) are used to identify the association state of the linker and the size of the resulting protein-RNA complex. We are currently working to biophysically characterize the structure, dynamics, and viral RNA binding ability of a mutation found in the Delta and Omicron variants: the R203M linker, which have been shown to enhance viral infectivity.

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